

## CASE REPORT

# Lipoid proteinosis: case report and review of the literature

## *Lipoido proteinosi: descrizione di un caso clinico e revisione della letteratura*

S. DI GIANDOMENICO, R. MASI<sup>1</sup>, D. CASSANDRINI<sup>4</sup>, M. EL-HACHEM<sup>2</sup>, R. DE VITO<sup>3</sup>, C. BRUNO<sup>4</sup>, F.M. SANTORELLI

Unit of Molecular Medicine, <sup>1</sup> Unit of Audiology, <sup>2</sup> Unit of Dermatology, <sup>3</sup> Unit of Pathology, IRCCS-Bambino Gesù Hospital, Rome; <sup>4</sup> Department of Paediatrics, University of Genoa and Neuromuscular and Neurogenetic Units, IRCCS-G. Gaslini, Genoa, Italy

## Key words

Larynx • Skin • Lipoid proteinosis • ECM1

## Parole chiave

Laringe • Cute • Lipoido proteinosi • ECM1

## Summary

Lipoid proteinosis is a rare autosomal recessive disorder, characterized histologically by infiltration of periodic acid Schiff-positive hyaline material into the skin, upper aerodigestive tract, and internal organs. Classical clinical features include skin scarring, beaded eyelid papules, and laryngeal infiltration leading to hoarseness. Moreover, the infiltrates in the tongue and its frenulum limit lingual movements and cause speech difficulties. Usually, the hoarse voice is present at birth or in early infancy, as the first manifestation. In more severe cases, diffuse infiltration of the pharynx and larynx might cause respiratory distress, at times requiring tracheostomy. The disorder has recently been shown to result from loss-of-function mutations in the extracellular matrix protein 1 gene on chromosome 1q21. The function of the protein extracellular matrix protein 1 gene is still unclear, although an important role in skin physiology and homeostasis has been hypothesized. In this report, the case is described of a 6-year-old girl with lipoid proteinosis. Histopathological examination of a laryngeal biopsy specimen showed massive deposits of eosinophilic, periodic acid Schiff-positive, and diastase resistant material in the lamina propria corroborating the clinical diagnosis of lipoid proteinosis. Molecular analyses in this patient also confirmed the clinical diagnosis. The proband was a compound heterozygote for a new small rearrangement (543delTG/ins15) in exon 6, and a nonsense mutation (Arg243Stop) in exon 7. Together with previously documented mutations in the extracellular matrix protein 1 gene, this study supports the hypothesis that exons 6 and 7 are the most common sites for extracellular matrix protein 1 gene mutations in lipoid proteinosis.

## Riassunto

La sindrome di Urbach-Wiethe (lipoido proteinosi, LP) è un raro disordine ereditato con modalità autosomica recessiva caratterizzato da un punto di vista istologico da infiltrazioni di materiale ialino positivo all'acido periodico di Schiff (PAS) nella cute, nel tratto aerodigestivo superiore e negli organi interni. Le caratteristiche cliniche classiche comprendono la comparsa di cicatrici della cute, di papule sui margini degli occhi, e di infiltrazioni nella laringe che provocano raucedine. Inoltre gli infiltrati nella lingua e nel frenulo limitano i movimenti linguali e causano difficoltà della parola. Nei casi più gravi, l'infiltrazione diffusa della faringe e della laringe possono essere causa di distress respiratorio, e a volte può essere richiesta la tracheotomia. Recentemente è stato dimostrato che il disordine è il risultato di mutazioni nel gene codificante per la proteina 1 della matrice extracellulare (ECM1) che mappa sul cromosoma 1q21. La funzione della proteina ECM1 non è ancora chiarita, tuttavia è stato ipotizzato che svolga un importante ruolo nella fisiologia e nell'omeostasi della cute. In questo lavoro, noi descriviamo le caratteristiche clinico-patologiche di una bambina di 6 anni di età con LP che coinvolge la cute, l'orofaringe e la laringe. L'esame istopatologico del campione di biopsia laringea ha mostrato cospicui depositi di materiale diastasi resistente, eosinofilo e PAS-positivo nella lamina propria corroborando la diagnosi clinica di LP. Anche l'analisi molecolare nella nostra paziente ha confermato la diagnosi clinica, mostrando una nuova mutazione eterozigote (543delTG/ins15) nell'esone 6 e la mutazione Arg243Stop nell'esone 7. Il confronto delle mutazioni ECM1 identificate in questo studio con quelle precedentemente riportate in letteratura ci permette di confermare che gli esoni 6 e 7 sono le regioni di ECM1 più comunemente colpite da mutazioni in LP.

## Introduction

Lipoid proteinosis (LP) (Urbach-Wiethe disease, MIM 247100) is a rare autosomal recessive genodermmatosis first described in 1929; fewer than 300 cases have been reported in the literature to date. Although LP occurs worldwide, ~25% of all reported cases have been observed in South Africa, where many of the patients are of Dutch or German ancestry<sup>1-3</sup>.

The clinical manifestations of LP are protean and may vary considerably between affected individuals<sup>2</sup>. The disease may display multiple systemic involvement, although the skin and mucosal membranes of the upper aerodigestive tract are those primarily affected. Onset is usually in early infancy, and signalled by a weak cry and hoarse voice due to laryngeal infiltration<sup>4</sup>. In more severe cases, this infiltration may lead to respiratory obstruction. Diffuse skin infiltration and thickening gradually occurs, resulting in papules and chicken pox-like scars. Infiltrates in the tongue and its frenulum limit tongue movements and cause speech difficulties. Calcifications in the temporal lobes or hippocampus have been reported, sometimes in association with neurological, psychiatric and neuropsychological sequelae<sup>5,6</sup>. Prognosis of LP is, nonetheless, relatively good *quoad vitam*, if not *quoad valetudinem*.

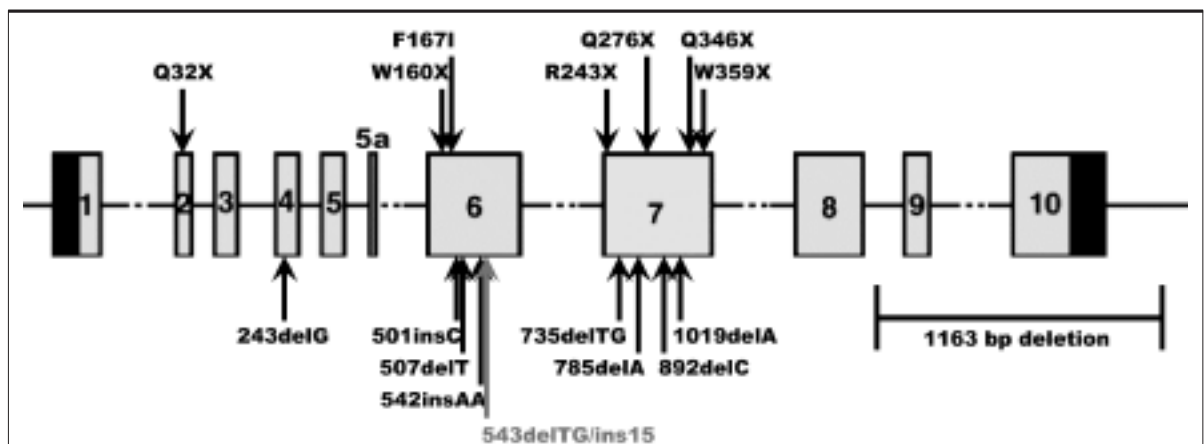
LP is characterized, histologically, by intercellular deposits of periodic acid Schiff (PAS)-positive hyaline material in the skin, mucous membranes, and internal organs. Loss-of-function mutations in the gene encoding extracellular matrix protein 1 (*ECM1*) on

chromosome 1q21<sup>7</sup> have been identified in LP; fewer than 20 variants have been reported (Fig. 1), most of which occur in single families. *ECM1* encompasses 11 exons spanning ~5,498 bp of the genomic sequence<sup>8</sup> and codes for a protein of 540 amino acids (ECM1a) (ECM1; 602201). Two distinct isoforms of different lengths have been described for *ECM1* depending on the presence of two alternatively spliced exons (exons 5a and 7) and both are expressed in the skin and upper respiratory tract, although ECM1a has a wider pattern of expression<sup>9</sup>. A third isoform, ECM1c, contains all 11 exons but has been detected in only two cancer cell lines<sup>10</sup>. The function of the protein ECM1 is still unclear, although an important role in skin physiology and homeostasis has been hypothesized<sup>8</sup>.

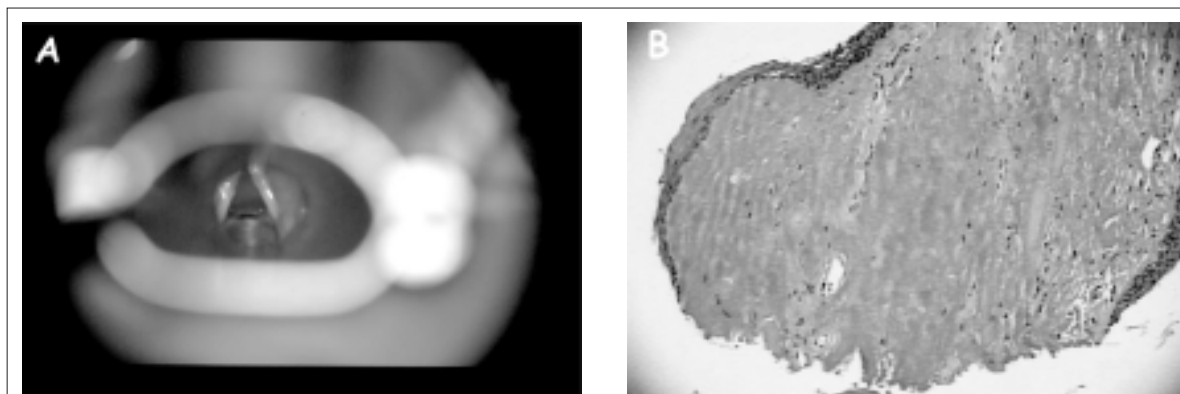
The present report focuses on clinical and genetic findings in an additional LP patient and summarizes pertinent data from the literature.

## Case report

A 6-year-old girl, born to unrelated parents, presented with progressive skin and mucous membrane changes since early childhood. The patient had developed hoarseness during the first few months of life and, at age 3 years, started to develop multiple warty papules on the dorsum of the hands, on the face and eyelid margins. Most papules were skin-coloured. Scarring was also evident. She had no history of frontal headache, seizures or visual distur-



**Fig. 1.** Morbidity map of *ECM1* gene. Illustration of position of new frameshift mutation (543delTG/ins15) (grey arrow) and previously identified mutations. Missense or nonsense mutations are depicted above and frameshift mutations below gene structure. Most mutations are nonsense or frameshift changes that occur within exon 6 or within differentially spliced exon 7.



**Fig. 2.** A). Deposits of homogeneous, proteinaceous material in vocal cords before surgery. B). Light microscopy examination of laryngeal biopsy from child with lipoid proteinosis showed massive deposits of homogeneous, eosinophilic, hyaline-like material in lamina propria, without inflammation. Material was strongly PAS positive, diastase resistant, revealing glycoproteic nature of the substance.

bance. The patient underwent indirect laryngoscopy, on account of severe dysphonia, which showed thickening of the vocal cords and hyaline deposits in the larynx, oral cavity and oropharynx. As proposed elsewhere<sup>11</sup>, the patient was submitted to a logopaedic programme; when this failed, the lesions were removed surgically, for temporary functional purposes. Light microscopy examination of laryngeal biopsy tissue showed massive deposits of homogeneous, eosinophilic, hyaline-like material in the lamina propria, without inflammation. The material was strongly PAS positive and diastase resistant, revealing the glycoproteic nature of the substance (Fig. 2). The most recent follow-up showed a slight improvement in the clinical appearance of the vocal cords, but little change in the dysphonia. The patient has recently started a new logopaedic programme, but prognosis has not changed.

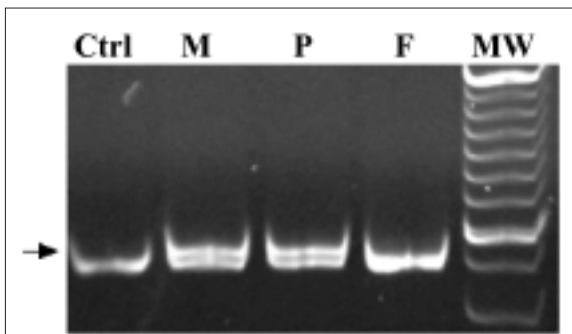
In order to obtain definitive molecular confirmation of LP in this patient, we analyzed the *ECM1* gene. Following parents' informed consent, genomic DNA was extracted from peripheral blood and the coding sequence of *ECM1* (mRNA Genbank accession number NM\_004425.2) was amplified by polymerase chain reaction (PCR) using intronic oligonucleotide primers, as reported elsewhere<sup>12</sup>. The PCR products were gel purified and directly sequenced using BigDye Terminator chemistry on an ABI 377 automatic sequencer (Applied Biosystems, Foster City, CA, USA). A small heterozygous deletion/insertion mutation in exon 6 was found. Subcloning into a PCR-compatible cloning vector (TOPO TA Cloning Kit, Invitrogen, Paisley, UK) and direct sequencing revealed a novel 543delTG/ins15, which results in frameshift and early translation termination at codon 187. By separation of PCR products, on acrylamide

gel electrophoresis, the heterozygous mutation in the patient and in blood DNA from the healthy mother was confirmed (Fig. 3). A previously documented heterozygous 726C>T (Arg243Stop) mutation<sup>12</sup> was detected on the paternal allele, and confirmed by primer extension using the ABI Prism SnaPshot Multiplex kit (Applied Biosystems, Foster City, CA, USA) (Fig. 4).

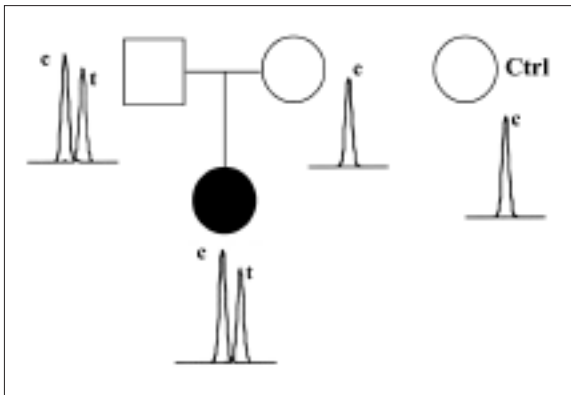
## Discussion

Although several reports of LP patients have appeared, mostly in the dermatology literature, emphasizing the typical dermatologic features, the first clinical manifestation of LP is usually progressive hoarseness caused by diffuse deposition of hyaline material in the mucous membranes of the vocal cords<sup>4</sup>. This hoarseness may be present at birth, as a weak cry, or develop later, within the first few years of life. The development of a hoarse voice was the first symptom noticed in our patient. Therefore, the importance of LP should not be underestimated by otolaryngologists and it should be included in the differential diagnosis of voice changes and hoarseness, in infancy and childhood<sup>13</sup>.

The skin of children with LP is highly susceptible to damage, being reflected in the appearance of chicken pox-like scars and yellowish papules. These lesions involve primarily the face and extremities and rarely appear elsewhere. Subsequently, infiltration of the skin may present as groups of warty plaques on the axillae and elbows. Beaded papules on the eyelid margins (moniliform blepharosis) are a characteristic finding in about two-thirds of patients. Our patient presented typical skin lesions, which appeared at approximately 3 years of age.



**Fig. 3.** Segregation analysis of 543delTG/ins15 in family of child with LP. PCR products spanning exon 6 and its flanking introns were resolved by 12% bisacrylamide-polyacrylamide gel electrophoresis and stained with ethidium bromide. A single PCR fragment sized 671-bp is obtained in wild-type individuals (F and Ctrl). By contrast, in individuals bearing heterozygous rearrangement (→) two PCR bands sized 684 and 671-bp are obtained (P and M). Ctrl, genomic DNA from normal control; M, mother; P, patient; F, father. MW is an 100-bp molecular marker size.



**Fig. 4.** Primer extension technique using ABI PRISM SnaPshot Multiplex kit (Applied Biosystems, Foster City, CA, USA) showed that 726C>T mutation (Arg243Stop) in *ECM1* was present on paternal allele in child with LP. Family pedigree is depicted and chromatograms of normal (c) and mutant (t) alleles are shown below. Ctrl, genomic DNA from normal control.

In clinical practice, LP is rarely a life-threatening condition<sup>14</sup>. Infiltration of the oral mucosa may lead to xerostomia and dysphagia. Other mucosal findings include thickening of the sublingual frenulum and tongue, limiting tongue movements and causing speech difficulties. Moreover, diffuse infiltration of the pharynx and larynx may cause respiratory distress, at times requiring tracheostomy. Our patient presented a speech impairment but no upper airway obstruction or dyspnoea. Hyaline deposits have been

described in the conjunctiva, cornea, trabeculum, and retina<sup>15</sup>. Corneal opacities or secondary glaucoma, due to infiltration in the trabeculum, may appear later. Deposition of hyaline material in the small bowel may lead to intestinal bleeding. Although autopsy studies have shown LP to be a generalized disorder with microscopic deposits of hyaline material in practically every organ, symptoms related to other viscera have not been described. Extracutaneous and extramucosal features may include neurological signs and symptoms, such as epilepsy, memory loss, and schizophrenic behaviour, sometimes in association with intracranial calcification in the temporal lobes or hippocampus, easily detected by brain computed tomography<sup>5,6</sup>.

Histologically, LP is characterized by disruption/duplication of the basement membrane and deposition of hyaline material at the level of the basement membrane (resulting in its thickening at the dermo-epidermal junction), papillary dermis, surrounding capillaries, and around adnexal epithelia, especially sweat coils. The hyaline material is eosinophilic, PAS-positive, and diastase resistant, indicating the presence of glycoproteins. Accumulations of type IV and V collagen occur around the blood vessels and appendages; type I and III collagen is reduced<sup>16-18</sup>. Our patient was not subjected to skin biopsy. However, histopathological examination of a laryngeal biopsy specimen showed massive deposits of eosinophilic, PAS-positive, and diastase resistant material in the lamina propria corroborating the clinical diagnosis of LP. Molecular studies ultimately confirmed the clinical diagnosis. The probanda was a compound heterozygote for a small rearrangement (543delTG/ins15) in exon 6, and a nonsense mutation (Arg243Stop) in exon 7. Both mutations lead to early protein truncation and likely loss of function. This finding is in keeping with data reported in the literature, which suggest that LP patients usually harbour mutations in exon 6 and, especially, 7 of *ECM1*. Recent cell biology studies have provided new data that further our understanding of the function of *ECM1* and that partially explain the disease phenotype in LP. *ECM1* has been reported to regulate endochondral bone formation, to stimulate proliferation of blood vessel endothelial cells, to promote angiogenesis, and to be involved in the control of epidermal differentiation<sup>19-21</sup>. However, clues to its physiological function in the dermis are now emerging. It has been demonstrated that *ECM1* is a secreted glycoprotein that binds to perlecan, the major heparan sulphate proteoglycan of the basement membrane, as well as to growth factors and fibrillar proteins<sup>10,22</sup>. Thus, *ECM1* may act as a “biological glue” in the dermis, helping to regulate basement membrane and interstitial collagen fibril macro-assembly and growth factor binding. Therefore, loss of *ECM1*,



within the dermis may have profound effects on dermal homeostasis, leading to the clinical features of skin infiltration and scarring. Meanwhile, lack of ECM1 within the epidermis may alter the normal pattern of keratinocyte maturation and differentiation and give rise to the clinical features of warty hyperkeratosis.

Some Authors have attempted to correlate genotype with clinical manifestations<sup>7,12</sup>. Nonsense or frameshift mutations in exon 7 lead to ablation of the ECM1a transcript, but not the shorter ECM1b transcript that normally lacks this exon and that is expressed in skin and upper respiratory tract. From a clinical point of view, it is suggested that the patients harbouring mutations in exon 7 could have a slightly less severe skin and respiratory tract phenotype compared to cases bearing mutations not affecting exon 7<sup>7,12</sup>. Our patient, despite the presence of nonsense mutation in exon 7, developed hoarseness during the first months of life and skin manifestations in early childhood. This finding favours the hypothesis that the isoform containing exon 7 (ECM1a) is of fundamental biological importance. Instead, no specific correlation between genotype and neurological manifestations has ever been shown<sup>12</sup>. We are unable to support the hypoth-

esis suggesting the lack of such a correlation since we cannot, at present, predict the evolution of the clinical syndrome in our patient or the possibility of her developing neurological complications. Furthermore, a detailed analysis of a large, inbred South African family demonstrated that individuals sharing the same genetic background and with an identical mutation can present vastly different phenotypes during disease progression<sup>3</sup>. Other genetic and epigenetic factors might be invoked.

The prognosis of LP patients is generally good despite the progressive nature of the disease until early adulthood. However, there is currently no effective therapy. Laser microlaryngoscopy, dissection of the vocal cords and excision of deposits may be performed to preserve or improve the voice. Respiratory obstruction is infrequent and rarely requires tracheostomy. Dermabrasion and chemical skin peeling can be performed in some cases. Approaches reported in the literature include oral steroids, dimethyl sulphoxide, intralesional heparin, and etretinate<sup>7</sup>. Irrespective of evolution of the disease and use of symptomatic treatment, it is important that parents of affected children be counselled concerning the risks of having other affected offspring.

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■ Address for correspondence: Dr. F.M. Santorelli, Ospedale Pediatrico Bambino Gesù, piazza Sant'Onofrio 4, 00168 Roma, Italy. Fax +39 06 68592024. E-mail: fms3@na.flashnet.it